

1 WHAT IS CLAIMED IS:

1 1. A method for immobilizing a polypeptide to a surface, wherein the
2 method comprises:

3 contacting a polypeptide which comprises an ester or thioester, with an
4 anchor molecule comprising a first nucleophilic group at a 2 or 3 position relative to a
5 second nucleophilic group,

6 wherein the ester or thioester undergoes a trans-esterification reaction
7 with the first nucleophilic group, thus forming an intermediate
8 compound in which the polypeptide is attached to the anchor molecule
9 through the first nucleophilic group; and

10 attaching the anchor molecule to a surface.

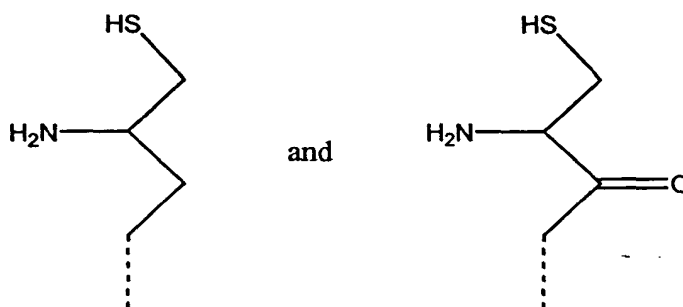
1 2. The method of claim 1, wherein the intermediate compound
2 undergoes an intramolecular rearrangement in which the second nucleophilic group on the
3 anchor molecule displaces the first nucleophilic group, thus forming a more stable bond
4 between the anchor molecule and the polypeptide.

1 3. The method of claim 1, wherein the polypeptide comprise a thioester.

1 4. The method of claim 1, wherein the anchor molecule comprises a 2-
2 aminonucleophile or a 3-aminonucleophile.

1 5. The method of claim 4, wherein the 2-aminonucleophile is a 2-
2 aminothiols.

1 6. The method of claim 5, wherein the anchor molecule comprises a
2 structure selected from the group consisting of:



3

1 7. The method of claim 1, wherein the anchor molecule is attached to the
2 surface prior to contacting the anchor molecule with the polypeptide.

1 8. The method of claim 1, wherein the anchor molecule is attached to the
2 surface after contacting the anchor molecule with the polypeptide.

1 9. The method of claim 1, wherein the anchor molecule comprises a
2 functional group that can be covalently linked to a molecule that is attached to the surface.

1 10. The method of claim 9, wherein the functional group is selected from
2 the group consisting of ketones, diketones, olefins, epoxides, aldehydes, reactive esters,
3 isocyanates, thioisocyanates, carboxylic acid chlorides, disulfides, sulfonate esters,
4 maleimide, isomaleimide, N-hydroxysuccinimide, nitrilotriacetic acid, activated hydroxyl,
5 haloacetyl, activated carboxyl, hydrazide, epoxy, aziridine, sulfonylchloride, acyl
6 hydrazines, trifluoromethyldiaziridine, pyridyldisulfide, N-acyl-imidazole,
7 imidazolecarbamate, vinylsulfone, succinimidylcarbonate, arylazide, anhydride,
8 diazoacetate, benzophenone, isothiocyanate, isocyanate, imidoester, aminooxy and
9 fluorobenzene.

1 11. The method of claim 1, wherein the anchor molecule comprises a tag
2 moiety that can be noncovalently bound to a molecule that is attached to the surface.

1 12. The method of claim 11, wherein the tag comprises a binding domain
2 which is derived from a polypeptide selected from the group consisting of glutathione-S-
3 transferase (GST), maltose-binding protein, chitin, cellulase, thioredoxin, avidin,
4 streptavidin, and green-fluorescent protein (GFP).

1 13. The method of claim 11, wherein the tag comprises a chitin binding
2 domain or a cellulose binding domain.

1 14. The method of claim 11, wherein the tag comprises a peptide that
2 comprises an amino-terminal Cys, Thr, or Ser.

1 15. The method of claim 1, wherein the polypeptide comprises a non-
2 natural amino acid.

1 16. The method of claim 1, wherein the ester or thioester is chemically
2 introduced onto the polypeptide.

1 17. The method of claim 1, wherein the ester or thioester is introduced
2 onto the polypeptide by chemical synthesis of the polypeptide.

1 18. The method of claim 1, wherein the polypeptide that comprises an
2 ester or thioester is obtained by:
3 expressing a chimeric gene that encodes a fusion protein which comprises:
4 the polypeptide and an intein, or a functional portion thereof, which is joined
5 to the polypeptide at a splice junction at the amino terminus of the intein, wherein the
6 carboxyl terminus of the intein lacks a functional splice junction; and
7 contacting the fusion protein with a nucleophilic compound which releases
8 the polypeptide from the intein at the splice junction and forms the polypeptide that
9 comprises a terminal ester or thioester.

1 19. The method of claim 18, wherein the nucleophilic compound is the
2 anchor molecule.

1 20. The method of claim 18, wherein the nucleophilic compound
2 comprises a peptide.

1 21. The method of claim 20, wherein the peptide comprises a serine,
2 threonine or cysteine at its amino terminus, the oxygen and sulfur of which are the
3 nucleophilic groups that undergo the transesterification reaction.

- 1 22. The method of claim 18, wherein the nucleophilic compound
2 comprises a thiol as the nucleophile.
- 1 23. The method of claim 18, wherein the intein is an Int-n of a split intein
2 and the anchor molecule comprises an amino acid sequence that comprises an Int-c of a split
3 intein, wherein the Int-n and the Int-c undergo an intein splicing reaction, thus attaching the
4 anchor molecule to the polypeptide.
- 1 24. The method of claim 23, wherein the Int-n is derived from a *dnaE-n*
2 gene and the Int-c is derived from a *dnaE-c* gene.
- 1 25. The method of claim 24, wherein the *dnaE-n* gene and the *dnaE-c*
2 gene are from a cyanobacterium species.
- 1 26. The method of claim 25, wherein the cyanobacterium species is a
2 *Synechocystis* species.
- 1 27. The method of claim 18, wherein the fusion protein is expressed *in*
2 *vitro*.
- 1 28. The method of claim 18, wherein the fusion protein is expressed *in*
2 *vivo* by introducing the chimeric gene into a host cell and incubating the host cell under
3 conditions conducive to expression of the fusion protein.
- 1 29. The method of claim 1, wherein the surface comprises a biochip.
- 1 30. The method of claim 29, wherein the biochip comprises a non-sample
2 surface and a plurality of sample portions that are elevated with respect to the non-sample
3 surface and each sample portion has attached thereto a single polypeptide species.
- 1 31. The method of claim 29, wherein the biochip comprises one or more
2 materials selected from the group consisting of silicon, plastic, gold, and glass.
- 1 32. The method of claim 1, wherein the surface comprises a microparticle.

1 33. The method of claim 1, wherein the polypeptide is placed in contact
2 with the surface using a microvolume dispenser that comprises:
3 a body; and
4 at least one vertical channel defined within the body, the channel being
5 defined by at least one passive valve;
6 wherein an interior surface defining at least one vertical channel is
7 hydrophobic.

1 34. The method of claim 33, wherein the dispenser comprises a plurality
2 of vertical channels defined within the body.

1 35. The method of claim 34, wherein the vertical channels are arranged as
2 an array.

1 36. An array of immobilized polypeptides attached to a surface, wherein
2 the array comprises at least a first polypeptide species and a second polypeptide species and
3 each of which polypeptide species are:
4 attached to a separate region of the surface;
5 attached to the surface in the same orientation; and
6 are folded in a secondary structure as required for a biological activity.

1 37. The array of claim 36, wherein each of the peptide species are
2 covalently attached to a surface-bound linker by a 2-aminonucleophile ester bond.

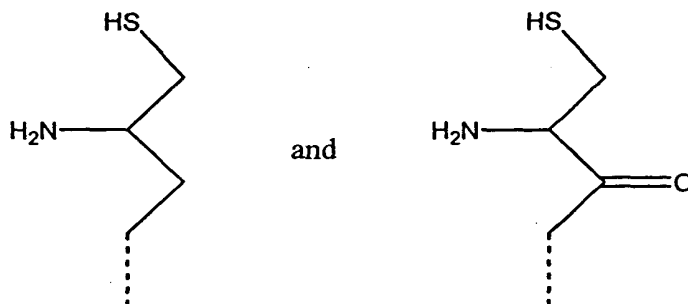
1 38. The array of claim 37, wherein the 2-aminonucleophile ester bond is a
2 2-aminothioester bond.

1 39. The array of claim 37, wherein the 2-aminonucleophile ester bond
2 undergoes an intramolecular rearrangement to form an amide bond.

1 40. The array of claim 37, wherein the linker is a non-peptide linker.

1 41. The array of claim 36, wherein the C-terminus of each of the
2 polypeptides is attached to the surface.

1 42. The array of claim 37, wherein the linker comprises a structure
2 selected from the group consisting of:



1 43. The array of claim 36, wherein the surface comprises a biochip.

1 44. The array of claim 43, wherein the biochip comprises a non-sample
2 surface and a plurality of sample portions that are elevated with respect to the non-sample
3 surface and each sample portion has attached thereto a single polypeptide species.

1 45. The array of claim 43, wherein the biochip comprises one or more
2 materials selected from the group consisting of silicon, plastic, gold, and glass.

1 46. An array of immobilized polypeptides attached to a surface which
2 comprises a plurality of surface regions, wherein each surface region has attached thereto a
3 polypeptide species and a polynucleotide that encodes the polypeptide species.

1 47. The array of claim 46, wherein the surface comprises a biochip.

1 48. The array of claim 47, wherein the biochip comprises a non-sample
2 surface and a plurality of sample portions that are elevated with respect to the non-sample
3 surface and each sample portion has attached thereto a single polypeptide species and a
4 polynucleotide that encodes the polypeptide species.

1 49. The array of claim 47, wherein the biochip comprises one or more
2 materials selected from the group consisting of silicon, silicon oxide, plastic and glass.

1 50. A method for screening a library of nucleic acids to identify a nucleic
2 acid that encodes a polypeptide having a desired activity, the method comprising:

3 expressing a plurality of fusion proteins, each of which is encoded by an
4 expression cassette that comprises:
5 a) a member of the library of nucleic acids;
6 b) an intein coding region; and
7 c) an open reading frame that encodes a polypeptide that is displayed on
8 a surface of a replicable genetic package;
9 wherein the fusion proteins are displayed on the surface of a replicable
10 genetic package; and
11 screening the replicable genetic packages to identify those that display a
12 polypeptide having the desired activity.

1 51. The method of claim 50, wherein the polypeptide encoded by the
2 library member is released from the fusion protein by contacting the phage with a
3 nucleophilic compound, which nucleophilic compound becomes attached to the polypeptide.

1 52. The method of claim 51, wherein the nucleophilic compound
2 comprises a compound that has a first nucleophilic group and a second nucleophilic group at
3 a 2 or 3 position relative to the first nucleophilic group.

1 53. The method of claim 52, wherein the nucleophilic compound is a 2-
2 aminonucleophile or a 3-aminonucleophile.

1 54. The method of claim 53, wherein the nucleophilic compound is a 2-
2 aminothiols or a 3-aminothiol.

1 55. The method of claim 51, wherein the nucleophilic compound
2 comprises a thiol or a hydroxyl.

1 56. A nucleic acid that comprises an expression cassette, wherein the
2 expression cassette comprises:
3 an insertion site at which a polynucleotide can be introduced into the
4 expression cassette;

5 an intein coding region, wherein the carboxyl terminus of the intein coding
6 region is mutated so that it does not function as a splice junction for intein-mediated
7 cleavage; and

8 an open reading frame that encodes a polypeptide that is displayed on a
9 surface of a replicable genetic package;

10 wherein the introduction of a polynucleotide at the insertion site results in an
11 open reading frame that encodes a fusion protein which comprises a polypeptide encoded by
12 the polynucleotide, which polypeptide is attached at its carboxyl terminus to an amino
13 terminus of the intein, and the surface-displayed polypeptide is attached to a carboxyl
14 terminus of the intein.

1 57. The nucleic acid of claim 56, wherein the expression cassette further
2 comprises a promoter.

1 58. The nucleic acid of claim 56, wherein the polynucleotide is a member
2 of a library of polynucleotides.

1 59. The nucleic acid of claim 58, wherein the library of polynucleotides is
2 a library of cDNA molecules, genomic DNA fragments, or recombination products.

1 60. A method for immobilizing a polypeptide to a surface, wherein the
2 method comprises:

3 contacting a polypeptide which comprises an ester or thioester, with an
4 anchor molecule comprising a first nucleophilic group at a 2 or 3 position relative to a
5 second nucleophilic group,

6 wherein the ester or thioester undergoes a trans-esterification reaction
7 with the first nucleophilic group, thus forming an intermediate
8 compound in which the polypeptide is attached to the anchor
9 molecule through the first nucleophilic group;

10 wherein said intermediate compound undergoes an intramolecular
11 rearrangement in which the second nucleophilic group on the
12 anchor molecule displaces the first nucleophilic group, thus

13 forming a bond between the anchor molecule and the
14 polypeptide; and
15 attaching the anchor molecule to a surface.

1 61. A method for immobilizing a polypeptide to a surface, wherein the
2 method comprises:
3 contacting a polypeptide which comprises an ester or thioester, with an
4 anchor molecule comprising a reactive group selected from the group consisting of a NH₂-
5 NH-R group and an aminooxy group
6 wherein R represents an anchor molecule,
7 wherein the ester or thioester reacts with the reactive group, thus
8 forming a compound comprising a polypeptide attached to the
9 anchor molecule through the reactive group.

1 62. The method of claim 61, wherein the polypeptide that comprises an
2 ester or a thioester are obtained by:
3 expressing a chimeric gene that encodes a fusion protein which comprises:
4 the polypeptide; and
5 an intein, or a functional portion thereof, which is joined to the polypeptide at
6 a splice junction at the amino terminus of the intein, wherein the carboxyl terminus of the
7 intein lacks a functional splice junction; and
8 contacting the fusion protein with a nucleophilic compound which releases
9 the polypeptide from the intein at the splice junction and forms the polypeptide that
10 comprises a terminal ester or thioester.

1 63. The method of claim 62, wherein the nucleophilic compound is the
2 anchor molecule.

1 64. The method of claim 62, wherein the nucleophilic compound
2 comprises a peptide.

1 65. The method of claim 64, wherein the peptide comprises a serine,
2 threonine or cysteine at its amino terminus.

1 66. The method of claim 62, wherein the nucleophilic compound
2 comprises a thiol as the nucleophile.

1 67. The method of claim 61, wherein the anchor molecule is attached to
2 the surface after contacting the anchor molecule with the polypeptide.

1 68. The method of claim 61, wherein the anchor molecule comprises a
2 functional group that can be covalently linked to a molecule that is attached to the surface.

1 69. The method of claim 68, wherein the functional group is selected from
2 the group consisting of ketones, diketones, olefins, epoxides, aldehydes, reactive esters,
3 isocyanates, thioisocyanates, carboxylic acid chlorides, disulfides, sulfonate esters,
4 maleimide, isomaleimide, N-hydroxysuccinimide, nitrilotriacetic acid, activated hydroxyl,
5 haloacetyl, activated carboxyl, hydrazide, epoxy, aziridine, sulfonylchloride, acyl
6 hydrazines, trifluoromethyldiaziridine, pyridyldisulfide, N-acyl-imidazole,
7 imidazolecarbamate, vinylsulfone, succinimidylcarbonate, arylazide, anhydride,
8 diazoacetate, benzophenone, isothiocyanate, isocyanate, imidoester, aminooxy and
9 fluorobenzene.

1 70. The method of claim 61, wherein the anchor molecule comprises a tag
2 moiety that can be noncovalently bound to a molecule that is attached to the surface.

1 71. The method of claim 70, wherein the tag comprises a binding domain
2 which is derived from a polypeptide selected from the group consisting of glutathione-S-
3 transferase (GST), maltose-binding protein, chitin, cellulase, thioredoxin, avidin,
4 streptavidin, and green-fluorescent protein (GFP).

1 72. The method of claim 70, wherein the tag comprises a chitin binding
2 domain or a cellulose binding domain.

1 73. The method of claim 70, wherein the tag comprises a peptide that
2 comprises an amino-terminal Cys, Thr, or Ser.

1 74. The method of claim 61, wherein the polypeptide comprises a non-
2 natural amino acid.

1 75. The method of claim 61, wherein the ester or thioester is chemically
2 introduced onto the polypeptide.

1 76. The method of claim 61, wherein the ester or thioester is introduced
2 onto the polypeptide by chemical synthesis of the polypeptide.

1 77. A kit for use in immobilizing one or more polypeptides containing an
2 ester or thioester to a surface of a substrate comprising:

3 an anchor molecule reagent for adapting said ester or thioester containing
4 polypeptide to said surface,

5 wherein said anchor molecule comprises a first nucleophilic group at a
6 2 or 3 position relative to a second nucleophilic group,

7 wherein the ester or thioester of said one or more polypeptides
8 undergoes a trans-esterification reaction with the first nucleophilic group, thus forming an
9 intermediate compound in which the polypeptides are attached to the anchor molecules
10 through the first nucleophilic group,

11 wherein said anchor molecule is adapted for attachment to said
12 surface of said substrate.

1 78. The kit of claim 77 further comprising:

2 a DNA vector for introducing said ester or thioester into said polypeptide,
3 said vector being adapted to receive a nucleic acid sequence encoding said polypeptide to
4 form a ester or thioester polypeptide expression vector for expressing said polypeptide as an
5 ester or thioester polypeptide having said ester or said thioester incorporated therein.

1 79. The kit of claim 77 further comprising:

2 a chemical agent for introducing into said polypeptide an ester or thioester.

1 80. The kit of claim 77 further comprising:

2 instructions for instructing a user to carry out the method of claim 1 using
3 said kit.

1 81. The kit of claim 77 further comprising:
2 a substrate for attaching said anchor molecules thereto for immobilizing said
3 polypeptides thereon.

1 82. The kit of claim 81, wherein said anchor molecule is supplied attached
2 to said surface of said substrate for later attaching said polypeptide thereto by a user.

1 83. The kit of claim 77, wherein said polypeptides are supplied with said
2 kit.

1 84. The kit of claim 83, wherein said polypeptides are supplied with said
2 kit pre-coupled with said anchor molecule(s).

1 85. The method of claim 77, wherein said substrate comprises a
2 microparticle.